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Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*)

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Abstract

Early developmental conditions can significantly influence the growth and survival of many animal species. We studied the consequences of exposure to corticosterone (CORT), a stress hormone, during the nestling stage on two behavioral traits (neophobia, social dominance) measured when the birds had reached independence. Nestling zebra finches (*Taeniopygia guttata*) were exposed twice daily to exogenous CORT via oral administration for a 12-day period up until fledging. Experimental CORT administration depressed nestling growth rates, confirming results previously obtained in this species. Our data on neophobic behavior revealed a significant interaction between sex and treatment, with CORT-dosed males showing reduced latencies to approach a novel object, while there was little effect of corticosterone treatment on female neophobia. There was no significant effect of age (30 or 50 days), however, there was a non-significant trend towards an interaction between treatment and age, with neophobia increasing with age in the CORT-dosed birds, but decreasing in controls. At 50 days of age previous exposure to corticosterone resulted in reduced success in competitions for a non-food-based resource (a perch) in both sexes. There were no effects of brood size on any behavioral traits measured here, but this may be due to the small range in brood size used. Our results show that elevated levels of stress hormones during postnatal development can have significant effects on important behavioral traits, i.e., neophobia and dominance. Moreover, they confirm the importance of rearing conditions in shaping adult phenotypes.

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Keywords: Stress hormones; Behavioral development; Evolution; Developmental stress; Phenotypic plasticity; Social dominance

Introduction

Developmental conditions can have significant effects on the expression of adult phenotypes (Bateson, 2001; Birkhead et al., 1999; Blount et al., 2003; Buchanan et al., 2003; Gil, 2003; Ketterson et al., 1996; Metcalfe and Monaghan, 2001; Moussaeu and Fox, 1998; Nowicki et al., 2002; Spencer et al., 2003, 2005; Verhulst et al., 2006). Such effects are not restricted to morphology, but extend to highly flexible traits such as behavior. For example, adverse environments during growth and development have been linked to adjustments in fear-related behavior (Gregus et al., 2005; Penke et al., 2001),

sexual signal expression (Blount et al., 2003; Buchanan et al., 2003; Gustafsson et al., 1995; Nowicki et al., 1998, 2002; Ohlsson et al., 2002; Spencer et al., 2003, 2004, 2005) and cognitive abilities (de Kloet et al., 2002; Kitaysky and Wingfield, 2003; Sandstrom and Hart, 2005) in adult animals. Many studies in adult birds have shown that plasma concentrations of corticosteroids can rise rapidly in response to a range of environmental perturbations (Pereyra and Wingfield, 2003; Silverin et al., 1997; Wingfield et al., 1998; Wingfield, 1994), however, until recently equivalent data in growing birds have been lacking. Recent studies have now shown that during postnatal development, a range of natural and experimental stressors, such as food restriction, aggression and handling can induce elevated circulating concentrations of stress hormones in nestlings of many altricial avian species (Blas et al., 2005; Bowerman et al., 2002; Kitaysky et al., 2001a, 1999; Love et al., 2003a,b; Saino et al., 2003; Sims and Holberton, 2000;

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Sockman and Schwabl, 2001). The existence of this physiological response in growing individuals poses the question whether exposure to stress hormones during development influences an individual's phenotype in the longer term, after the stressful circumstances have subsided.

Exposure to elevated levels of stress hormones during development has been shown to modify the physiological response to experimentally induced stress in adulthood, in many cases elevating maximal levels or prolonging levels over time (Anisman et al., 1998; Hayward and Wingfield, 2004; Penke et al., 2001; Parfitt et al., 2004). It is thought that this effect may be linked to a change in glucocorticoid receptor expression during development and thus regulation of stress hormone levels later in life, as well as the development of other components of the underlying neuroendocrine stress axis (Anisman et al., 1998; Sapolsky and Meaney, 1986). Early exposure to stress hormones may therefore play a role in 'programming' the neuroendocrine stress axis, shaping the 'hormonal phenotype' of the animal, with consequences for both behavioral and physiological responses to a variety of stressors in later life. In support of this hypothesis, developmentally mediated variation in the stress response has been linked to adjustments in some aspects of fear-related behavior and cognitive abilities in adult animals (de Kloet et al., 2002; Gregus et al., 2005; Kitaysky and Wingfield, 2003). Fear-related behaviors, which can have significant fitness effects, can be quantified in a standardized manner, by recording the behavioral responses of an animal to a novel object or environment.

Many birds are social foragers that live in large flocks, allowing more efficient foraging and greater individual energetic gains (Powell, 1974) and this is especially true during the non-breeding season and during early independence in many species including the zebra finch (Zann, 1996). During this activity dominance hierarchies are established and maintained through repeated pairwise interactions, where a dominant bird displaces a subordinate. These aggressive interactions can provoke large increases in individual stress hormones such as corticosterone in birds (Wingfield, 1994), in both the dominant and subordinate birds (Creel, 2001). Dominant birds often gain an advantage over subordinates through increased access to better or safer food or safe perching places, however, elevated social status can also carry significant costs (Verhulst and Salomons, 2004). Being able to compete for a range of resource types is therefore vital to ensure survival and maximize fitness, making competitive ability an essential part of an individual's behavioral phenotype.

In this study we investigated the effects of experimental exposure to the stress hormone corticosterone (CORT) during the latter half of the nestling period on neophobic behavior and competitive ability in later life in the altricial bird species zebra finch (*Taeniopygia guttata*). The study utilized genetically related sibling pairs of birds, where one received oral administration of small amounts of CORT between the ages of 7 and 18 days post-hatching and the other received only the carrier solution (peanut oil) during this period. Birds were then tested after nutritional independence to examine two aspects of

their behavioral phenotype: their response to novel objects and competitive ability.

Methods

Corticosterone manipulation

Adult zebra finches were randomly paired ($n=20$ pairs) and housed at the Biological Centre at the University of Groningen in $80 \times 40 \times 40$ -cm cages equipped with nestboxes and nesting material (hay). The birds were maintained at a temperature of 20–24°C and a photoperiod of 14L:10D. Breeding pairs were provided with a commercial tropical seed mixture (Teurlings, Dordrecht, The Netherlands), water, shell grit, and cuttlefish bone *ad libitum*. Pairs were also given egg food 3 times per week, until their offspring hatched when they received supplementary egg food daily. Upon hatching first and second hatched nestlings in each nest were individually marked by colored nail polish to each bird's toes. Brood sizes were standardized to either 3 or 5 nestlings (population range, 2–6 nestlings) by adding or removing nestlings at 2 days of age (additional nestlings in a nest were aged within 1 day of foster siblings), ensuring that two siblings remained in each nest that were genetically related to the parents. When nestlings reached 7 days of age experimental treatments began. First and second hatched chicks were assigned to one of two treatments: corticosterone administration or control, counterbalancing across broods for chick hatch order to control for any potential differences in basal corticosterone levels (Schwabl, 1999). Following Spencer et al. (2003), birds in the corticosterone group were dosed 0.0125 mg of corticosterone daily, dissolved in peanut oil (concentration 0.25 mg/ml) via two 25 μ l doses (at least 6 h apart) from the age of 7–18 days of age. This dosing schedule would have meant that birds experienced a reduced physiological dose with age as they increased in mass; a mass-scaled approach would have allowed the effective dose to remain constant. Control birds were dosed with the same amount of the peanut oil carrier solution at the same time as their sibling. All manipulations ceased at the age of 18 days. All birds had their mass recorded at 7, 12, 18 and 30 days of age. The sex of each bird was determined via the presence of sexually dimorphic adult plumage, which begins to appear after 35 days of age. All procedures were carried out following peer review and approval from the ethical committee for animal experiments of the University of Groningen (under license D4299).

Hormonal assay

To assess the effects of stress hormone administration on circulating levels of corticosterone over time blood samples (<100 μ l) were collected from 12-day-old birds at 0, 10, 30 or 120 min after administration of either corticosterone or peanut oil. Blood was collected in heparinized capillary tubes after puncture of the brachial vein with a 25-gauge needle, centrifuged and the plasma stored at -20°C for later hormone assay. Due to the small size of juvenile zebra finches each bird was bled once only. Samples were useable from 4 birds in each time period and each treatment (total $n=32$), this reduction in sample size was due to the difficulty in obtaining enough blood from such small birds. Corticosterone concentrations were measured after extraction of 20 μ l aliquots of plasma in diethyl ether, by radioimmunoassay (Maddocks et al., 2001; Buchanan et al., 2003) using anti-corticosterone antiserum code B21–42 (Endocrine Sciences, Tarzana, CA) and [1,2,6,7- ^3H]-corticosterone label (Amersham, UK). All birds showed corticosterone levels above the detection limit for this assay. The extraction efficiency was 70–91%. The assay was run with 50% binding at 1.48 ng/ml, and the detection limit for 7.3 μ l aliquots of extracted plasma was 0.31 ng/ml. All samples were run in duplicate in a single assay.

Behavioral measurements

Neophobic behavior

Neophobic behavior was investigated twice, once around the time of nutritional independence (27–35 days of age) and again when birds were fully independent from their parents (50–60 days of age). The same methodology was used for each trial. Each bird was removed from their home cage, which they

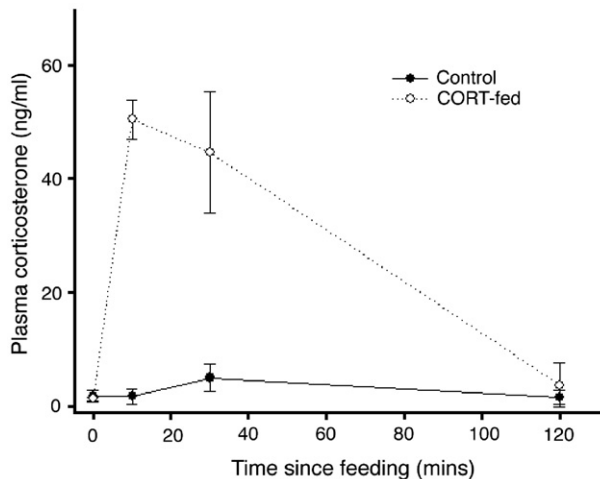


Fig. 1. The effect of corticosterone and peanut oil administration on plasma concentrations of corticosterone (CORT). Dashed line represents response of birds ($n=16$) following oral administration of corticosterone ($25 \mu\text{l}$ of 0.25 mg ml^{-1} concentration) and solid line shows responses of birds fed peanut oil alone ($n=16$).

shared with their parents and siblings and placed alone in a test cage otherwise identical to their home cage for a period of 10 min to allow acclimatization. The order in which each treatment underwent the trial was counterbalanced across sibling pairs and all data were recorded blind to treatment. The cage was equipped with two wooden perches and *ad libitum* food and water supplies (identical to the home cage). All trials were carried out in acoustic and visual contact with unrelated conspecifics to reduce any potential abnormal behavior. After the initial 10-min acclimatization period birds were subjected to two disturbances, the order of which was also counterbalanced across sibling pairs. Behavioral responses to a novel object were determined by placing a small toy moose (trials at 27–35 days) or a green furry ball (trials at 50–60 days) on a perch in the cage, allowing enough space for the bird to perch next to it if required. The following behaviors were then recorded over a 10-min period: latency to first landing on perch with novel object, total amount of time spent on the perch with the novel object and the amount of locomotion following disturbance (only during trials at 50–60 days). Locomotion was quantified by counting the number of times an individual moved position, e.g., from one perch to another or from the perch to the floor, and vice versa over the trial period. To control for the possible effects of human disturbance of placing the novel object on the perch we conducted a test to determine the effects of the observer placing his/her hand within the cage and touching the perch upon which the novel object was to be placed. After this disturbance the same behaviors were recorded as above for 10 min. This was done for all birds in the study and the data were used to scale the behavioral responses to the novel object (see Statistical analysis section below).

Dominance and competitive ability

We determined the ability of each bird to compete for a non-food resource (a perch) against a single competitor of a similar age (within 3 days) and mass (within 1 g) when birds were between 50 and 60 days of age. Each member of a sibling pair competed against the same individual, where possible, unless the sibling pair members were of different sexes. In this case each pair member competed against a bird of the same sex from the same nest as their sibling's competitor. Tests were carried out in a cage identical to their home cage which was equipped with a central divider. On each side of the divider a small perch was situated that was large enough for only one bird to perch on (5 cm in length). The order in which corticosterone-dosed and control siblings underwent this test was counterbalanced and all data were collected blind to treatment. The competitor and experimental bird were weighed and then placed in separate halves of the test cage in acoustic but not visual contact with each other and allowed to acclimatize for a 10-min period. All tests were carried out in acoustic and visual contact with conspecifics. All birds were observed to sit

on their perch within approximately 3 min of starting the acclimatization period and all were in this position at the start of each trial. The central divider was then removed temporarily and the experimental bird was allowed to fly into the competitor's cage, whereupon the divider was replaced, leaving the two birds to compete for the use of a single perch. Invariably removal of the divider caused the competitor to fly from the perch. The two birds were then observed for a 15-min period and for both birds the following behaviors were recorded: number of times on the perch, number of times supplanted by the other bird, total amount of time spent on perch, number of initiated aggressive interactions and number of unsuccessful attempts to gain access to the perch. To aid identification birds were color ringed, however, sibling pairs were ringed in the same color and all colors used (Orange (experimental birds), Purple (competitors)) are considered neutral with respect to aiding competitive ability or attractiveness (Burley, 1982, 1988).

Statistical analysis

All data were analyzed using Linear Mixed Models (SPSS, version 13.0), with cage of birth entered as random effect. For the analysis of neophobic behavior data from the two trials were combined to perform a repeated measures analysis with individual nested within cage of birth entered as an additional random factor. Fixed variables in each model were: brood size (3 or 5), sex and treatment (control or corticosterone). In addition age (trial 1 or 2) was added as a factor in the neophobia analysis and mass was entered as a covariate in the competitive ability model. Stepwise deletion of non-significant terms was employed. Relative measures were used as the dependent variables in the neophobic behavior models to control for the effects of disturbance on individual responses (e.g., latency to approach novel object perch – latency to approach perch after disturbance). Similar measures were used in the competitive ability models as all dependent variables were relative to the competitor's behavior (e.g., No. of times on perch by experimental bird – No. of times on perch by competitor). All residuals were checked for normality and transformed where necessary.

Results

Corticosterone titer and nestling growth

Plasma corticosterone levels increased significantly following oral administration (interaction: $F_{3,22} = 55.92$, $p < 0.001$;

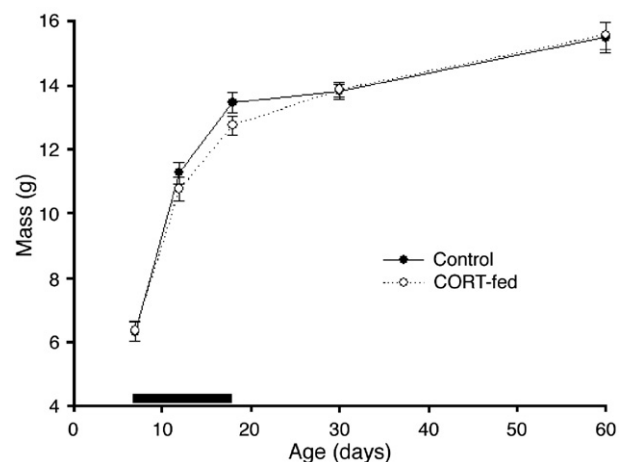


Fig. 2. The effect of corticosterone administration on growth rates in nestling zebra finches. The dashed line represents birds from the corticosterone group ($n=20$), solid line represents control birds ($n=20$). *Indicates ages where significant differences between the two treatment groups were found (Linear Mixed Model (REML) analysis $p < 0.05$). The bar on the x-axis represents the period of CORT administration.

Fig. 1). Levels peaked 10 min post administration, remained high until 30 min but were at basal levels by 120 min after administration. Thus birds would have experienced elevated levels for 1 to 4 h in a 24-h period. Levels in control birds did not change over time (Fig. 1). Exposure to elevated corticosterone levels reduced nestling growth rates: corticosterone-dosed birds were significantly lighter than controls at day 12 and day 18 post-hatching ($F_{1,18.2}=12.18$, $p=0.003$; $F_{1,18.5}=31.91$, $p<0.001$, respectively; Fig. 2). However, by days 30 and 60 there was no difference in the mass of birds in the two treatments ($F_{1,17.9}=0.96$, $p=0.34$; $F_{1,19.5}=0.35$, $p=0.56$, respectively; Fig. 2). There were no effects of sex or brood size manipulation on mass changes and no interactions between any of the fixed variables ($0.14<p<0.91$).

Neophobic behavior

Birds exposed to elevated corticosterone during postnatal development showed significantly reduced latencies to approach a novel object ($F_{1,52.6}=4.99$, $p=0.03$). Although the experimental effect was significant for the sexes combined, further analysis revealed that there was a significant interaction between treatment and sex ($F_{1,51.5}=4.82$, $p=0.03$). The overall effect can be attributed to the males only (Fig. 3). Females showed a similar response to a novel object being placed on the perch irrespective of treatment group in both trials. There was no effect of age (trial 1 versus 2; $F_{1,56.2}=0.33$, $p=0.57$) on neophobia, although there was a non-significant trend towards an interaction between treatment and age ($F_{1,52}=3.49$, $p=0.07$). The total amount of time spent on the novel perch within the trial period was not affected by treatment, sex or age alone ($p>0.44$), however, there was a significant interaction between treatment and sex ($F_{1,68.7}=4.78$, $p=0.03$), where CORT-dosed females showed a reduced amount of time on the perch compared to controls ($t=2.12$, $p=0.04$), and males were not affected by the treatment ($t=1.25$, $p=0.25$). There was also a non-significant trend towards a three-way interaction between treatment, sex and age ($F_{1,49.2}=2.47$, $p=0.07$). There were no effects of treatment or sex on the time spent on locomotion (measured in second experiment only; $0.16<p<0.89$). There were also no effects of (manipulated) brood size on any aspect of neophobic behavior measured here ($p>0.3$).

Dominance and competitive ability

Birds that had experienced elevated corticosterone during development exhibited reduced competitive ability compared to controls when aged between 50 and 60 days of age (Figs. 4a–d), showing lower numbers of successful perches ($F_{1,17.9}=4.12$, $p=0.05$), shorter times spent on the perch ($F_{1,17.8}=6.38$, $p=0.02$), higher levels of being supplanted from the perch ($F_{1,17.6}=9.56$, $p=0.004$) and a larger number of unsuccessful perches ($F_{1,17.6}=9.08$, $p=0.005$). Sex did not significantly affect competitive ability ($0.06<p<0.50$). There were also no significant interactions between treatment and sex ($0.1<p<0.25$). There were no effects of treatment on aggressive behavior ($F_{1,17.6}=0.03$, $p=0.95$). There were also

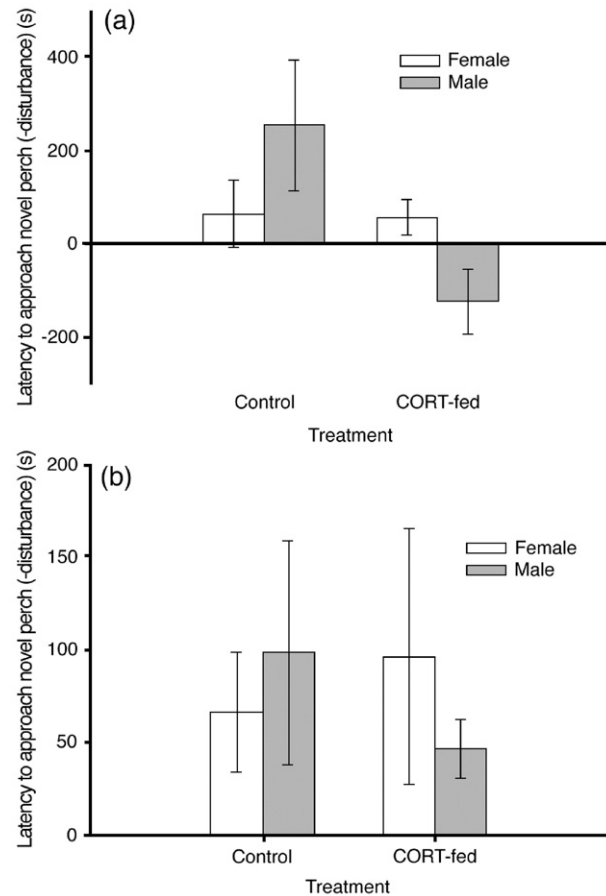


Fig. 3. The interaction between corticosterone treatment and sex in relation to the latency to approach a novel object at (a) 30 days post-hatching and (b) 50 days post-hatching. Data shown represent the mean difference (\pm SE) between the latency to approach the perch with a novel object and the latency to approach the perch after a disturbance. Sample sizes: control male=9, female=11; CORT-dosed male=10, female=10.

no effects of brood size or mass on any of the behavioral traits measured here ($0.19<p<0.87$).

Discussion

Our results clearly demonstrate that exposure to corticosterone during postnatal development can have significant delayed effects on behavior, which can affect neophobic responses and competitive ability at important life history stages. While previous work has provided evidence for such long-term effects of early exposure to stress, especially in mammals, this study has taken a novel approach to reveal a causal link between hormone exposure and behavior by directly manipulating corticosterone levels in birds. In rodents postnatal stress in the form of maternal separation, associated with significant reductions in growth rates as seen in this study and changes to the HPA stress response, can result in increased startle responses, increased anxiety behaviors and reduced cognitive abilities later in life (Anisman et al., 1998; Chung et al., 2005; Levine, 2005; Parfitt et al., 2004; Penke et al., 2001; Sandstrom and Hart, 2005). While less attention has been paid to these

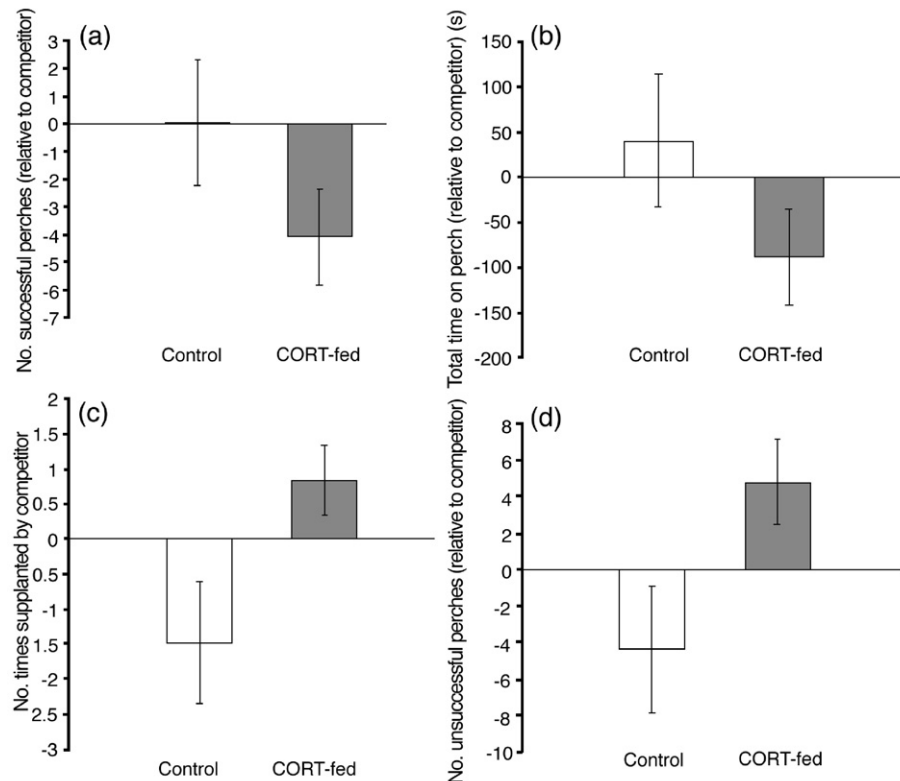


Fig. 4. The effect of postnatal exposure to corticosterone on (a) the number of successful perches made during observation period, (b) the total amount of time spent on perch, (c) the number of times an individual is supplanted by a competitor and (d) the number of unsuccessful perching attempts made during the observation period. All measures have been standardized relative to competitor behavior in each trial and show mean \pm SEM ($n = 20$ pairs of birds).

effects in avian species, there is some evidence that early pre- and postnatal conditions can influence later behaviors in a similar way to those seen in mammals (Hayward and Wingfield, 2004; Kitaysky and Wingfield, 2003) and the results of this study confirm the influential effects of adverse developmental conditions. It has been established that the physiological response to acute stress, that may underlie several of these changes in behavior can be significantly modified by early experiences, with stressful conditions promoting elevated peak levels and/or a prolongation of high circulating levels of corticosterone in later life (Hayward and Wingfield, 2004; Parfitt et al., 2004; Penke et al., 2001; Valee et al., 1999). Siblings in this study that underwent corticosterone treatments may have undergone these alterations to their physiology modifying the hormonal phenotype and their behavioral responses to the experimental stressors used in this study. However, further work will be required to determine the physiological basis for the results found here.

Our results also revealed a sex-specific pattern in the effects of developmental stress on neophobia in young birds, revealed by an interaction between treatment and sex. While females exhibited latencies to approach the perch following the introduction of the novel object, corticosterone treatment had no effect on this response. However, CORT-dosed females did show reduced perching time on the novel perch. Males were sensitive to postnatal corticosterone levels, with developmentally stressed males exhibiting significantly reduced latencies to

approach the novel object, that were in many cases lower than their response to a low level of disturbance and elevated perching time on the novel perch. Sex differences in response to nutritional stress during development were previously noted in zebra finches (de Kogel, 1997; Martins, 2004; Verhulst et al., 2006), but in the other direction, with females being more susceptible than males. Bowman et al. (2004) have shown that pre-natal stress can result in longer latencies in an open field test of neophobia in female rats. Male rats showed lower levels of neophobia in open field tests, but had reduced cognitive abilities in later life. This may suggest that pre- and postnatal conditions could have differential effects on behavior (Valee et al., 1999). One further explanation for the severe change in male behavior in our study may be that the very short latencies exhibited (compared to those following disturbance) do not reflect a reduction in fear-related behavior, rather they may be a side-effect of an elevated stress response, promoting increased startle responses or locomotion. It is well known that one behavioral outcome of the physiological stress response is elevated locomotion activity, thought to aid predator escape or foraging activities (Wingfield and Kitaysky, 2002; Wingfield et al., 1998) and postnatal stress has been associated with increased locomotion in response to novelty in mammals (Barbazanges et al., 1996). We found no effect of our treatment on locomotion activity in the second neophobia trial; however, this was not quantified in the first trial where the behavior was exhibited most strongly.

Competitive ability to gain access to a non-food resource was significantly affected by early exposure to corticosterone in this study. A range of captive studies have suggested that agonistic or competitive interactions can cause large and persistent increases in stress hormone secretion, especially where these interactions are unpredictable events (Creel, 2001). In this study birds were presented with such an unpredictable event, faced with an unfamiliar competitor for an important non-food resource in relatively unfamiliar surroundings (removed from natal group). It is therefore likely that stress hormone levels were elevated in both competitors and experimental birds. However, if early exposure to corticosterone had influenced the nature of the stress response, as suggested previously (Hayward and Wingfield, 2004; Parfitt et al., 2004; Penke et al., 2001; Valee et al., 1999), this may account for the treatment differences seen within sibling pairs. Elevated peak levels or faster rising levels in previously stressed birds could have increased locomotion and heightened the startle response, resulting in a higher number of perching attempts and higher levels of displacement events. However, we currently have no data on the long-term effects of our treatments on the physiological response to stress and more work is required to elucidate the proximate mechanism underlying these results. The ability of an individual to compete with conspecifics or access to food and non-food resources can have significant fitness-related and survival consequences, and previous work in a natural population of great tits (*Parus major*) has linked early life conditions to the ability to gain high quality breeding territories later in life (Verhulst et al., 1997). The results of our study suggest that one potential mechanism underlying Verhulst et al.'s finding could be differential exposure to stress hormones during postnatal development, however, further work would be required to determine the validity of this proposal.

Developmentally stressed birds exhibited reduced growth rates during the experimental period up until fledging (day 18 post-hatch), however, they also displayed a period of compensatory growth after fledging, reaching similar masses to controls by nutritional independence (age 30 days). These results confirm previous effects found in this species (Spencer et al., 2003). Periods of 'catch-up' growth can have significant deleterious effects on adult phenotypic traits, including reduced body size or organ size (Metcalf and Monaghan, 2001). While competitors were matched for mass in this study and siblings showed no differences in mass prior to the experiment, it is possible that developmentally stressed birds differed in traits not measured here, e.g., skeletal measurements or muscle mass. Whatever the underlying mechanism may be, the data presented here provide evidence that early exposure to stress hormones can mediate social dominance in later life, having detrimental effects on competitive ability, with potentially important later effects on fitness and survival.

The administration of exogenous corticosterone resulted in significant elevations of corticosterone in the plasma within 10 min of dosing. These levels remained high until 30 min post dosing, but dropped to basal levels within 120 min. There is growing evidence that nestling birds respond to stressful situations by elevating corticosterone levels over a similar

timescale as the one imposed by this study (Blas et al., 2005; Love et al., 2003a,b; Sims and Holberton, 2000). The levels of hormone experienced by these birds represent the high end of what altricial nestlings are known to experience following standardized stress protocols (Blas et al., 2005; Bowerman et al., 2002; Kitaysky et al., 2001a, 1999; Love et al., 2003a,b; Sims and Holberton, 2000; Sockman and Schwabl, 2001). However, the nature of our protocol means that birds would only have experienced these levels for only 1–4 h in a 24-h period. Several studies have shown high responses to stress in a proportion of nestlings in the latter stages of development (Blas et al., 2005; Bowerman et al., 2002; Kitaysky et al., 2001a, 1999; Love et al., 2003a,b; Saino et al., 2003; Sims and Holberton, 2000; Sockman and Schwabl, 2001). While we assert that our protocol resulted in biologically relevant elevations of corticosterone, it is likely that our birds are exhibiting larger or different effects than might be seen with lower doses of stress hormones. To fully understand the influence of corticosterone on long-term behavior we must therefore determine the range of effects gained from a range of developmental environments that differ in severity.

It is interesting that previous work on avian species has provided evidence for a potentially advantageous role for elevated stress hormones during the postnatal period. Kitaysky et al. (2001b) and Kitaysky and Wingfield (2003) have shown that experimentally increasing basal corticosterone levels in juvenile seabirds resulted in significant increases in begging rates. This was interpreted as an adaptive response to food stress by the chicks to gain access to more resources from the provisioning parents, and indeed chicks with experimentally amplified begging rates received food at higher rates, a relationship seen in many avian species (Iacovides and Evans, 1998; Kilner, 1995; Royle et al., 2002; Saino et al., 2000). Further work by Kitaysky and Wingfield (2003) has also suggested that this short-term advantage may be traded-off against a longer term cost. Our results concur with this hypothesis and suggest that although competitive ability may be enhanced by corticosterone during the nestling phase (Kitaysky and Wingfield, 2003; Spencer and Verhulst, unpublished data), this advantage is lost following independence.

In summary this study provides evidence in favor of the hypothesis that early developmental conditions can exert influence on long-term behavioral traits, and individual hormonal phenotypes. Further work in this area is required for us to determine the adaptive significance of these behavioral alterations and their importance for long-term survival and fitness.

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